Seed Bank Methodology Notes
JFSP Project 7-1-2-04
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Seed Bank Collection and Determination

Seed bank collection was done in late October-early November when soil seed reserves were most replete with available annual and perennial species. At the pinyon-juniper site, samples were obtained at 5-m intervals (5, 10, 15, 20, 25) along the west and east side of each vegetation plot (Figure 1). A sample consisted of a composite of two smaller sub-samples taken from around the perimeter of a 1-m quadrat. At the shrub-grassland site, samples were composited from soil taken from just outside each 2 m plot (Figure 2). To help overcome the spatial anomalies inherent in belowground seed distribution, collection of numerous, small samples was preferred to taking only a few large samples (Bossuyt et al 2007). However, the tendency of seeds to be clumped around a parent plant can still lead to an underestimation of species composition when samples fall within seed scarce regions between plants (Bigwood & Inouye 1988).

Initial samples were collected by pressing a tin soil canister (height 4.4 cm, diameter 6.0 cm) into the ground to a depth of 3.0 cm (85 ml sample). A metal spatula was then inserted underneath the canister to aid in the removal of a complete sample. Sampling was restricted to the top 3 cm of the soil as previous studies have found few seeds present below 2-3 cm in desert soils (Price & Reichman 1987; Ferrandis et al 2001; Kemp 1989). Obstructions such as rocks and woody debris (exceeding 1 inch in diameter) were picked up (with any external soil being brushed into the canister) and placed to the side. Standing vegetation was also avoided. Samples were then placed into a labeled bag and transported back to Flagstaff, AZ where they were placed outside in sealed, plastic containers for 2-3 months in order to vernalize the seeds. Outside conditions ranged from below freezing to 5° C. Samples were later brought into the greenhouse to be processed for the seed bank emergence portion of the study. The greenhouse was not kept at a constant temperature and ranged from a low of 5° C to a high of 20° C in during the winter months and 10° C to 30° C in the summer. No artificial lights were used.

The contents of the seed bank were ascertained using the emergence method standardized by the US Geological Survey, Western Ecological Research Center. These protocols are based on earlier methods used in the Great Basin (Young et al. 1969; Young and Evans 1978; Young et al. 1981), but were modified to capture annual plants found in the Mojave Desert (T. Esque et al. unpublished data). Many of these same annuals proliferate at our sites as well. Soils were brought out of storage, air-dried and ran through a 2 mm mesh sieve. Stones and organic debris were discarded after first removing any adhering soil. A one-half cup of the sifted sample was then mixed with one-half cup of Vermiculite to increase water retention. Each mix was placed in a 6 inch bulb pot lined with synthetic weedblock fabric. Pots were randomly placed on greenhouse benches and watered. Seedlings were identified, tallied and plucked as they emerged. Given that distinguishing between *B. tectorum* and *B. rubens* can be difficult at the seedling stage, they were collectively identified as *Bromus.* This process continued until germination had mostly ceased (4-6 weeks). The soil mixtures were allowed to dry out for 2-3 weeks followed by a second watering phase (3-4 weeks). This pattern was repeated two more times

with potassium nitrate (50 ml per pot/0.01 M solution) being added at the beginning of the third phase (2-3 weeks) and Gibberellic acid (50 ml per pot/$6.5x10^{-4}$ M solution) added at the beginning of the fourth phase (2-3 weeks). The dry-down period approximates natural moisture fluctuations necessary for germination to occur in some desert species (Baskin and Baskin 2001; Meyer et al. 2007). The chemical additives were included due to their previously documented ability to stimulate germination in perennial species (Jones and Nielson 1992; Bell et al. 1995; Baskin and Baskin 2001). Nomenclature for all emerging species followed USDA, NRCS (2009).

O**bservations**

Collection methods for this project were sufficient to capture the majority of annual species present at the site. However, to better represent perennial species, it would probably be necessary to collect additional samples from each plot. Timing of collections should also be adjusted to account for monsoonal moisture. In this study, we found that by postponing sampling until after the monsoons a large percentage of many annual species, especially *Bromus tectorum* (cheatgrass), had already emerged from the soil seed bank. However, sufficient seed remained to conduct credible analyses.

The small soil samples and pots were adequate for tallying emergence, however it was necessary to water daily and mortality of seedlings was often high during periods of hot weather. Larger samples and pots would help retain moisture and reduce seedling death. The majority of the soil samples had a moderate to high clay content. As a result, the vermiculite tended to sort to the surface of the samples rather than staying mixed in with the soil. A heavier type of amendment such as pearlite, would have been a better choice. Finally, the additions of giberellic acid and potassium nitrate did not appear to encourage germination of perennial species. However, a side study conducted by an undergraduate student (Jerry Elian) did find that potassium nitrate increased germination in several annual species. Still, if time is limited, we recommend that addition of these chemicals be dropped from the protocol.

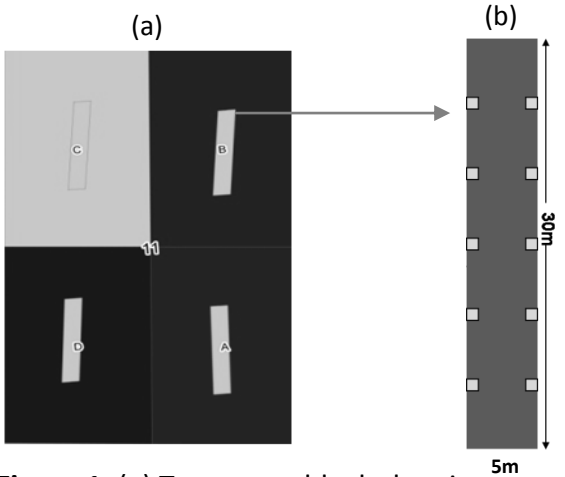

Figure 1. (a) Treatment block showing 5x30m plots surrounded by 15-m buffers. Each block contains 4 plots with a random treatment assignment (control, seeded, herbicide, seeded & herbicide). (b) Close-up of plot with 1-m squares showing soil sampling locations.

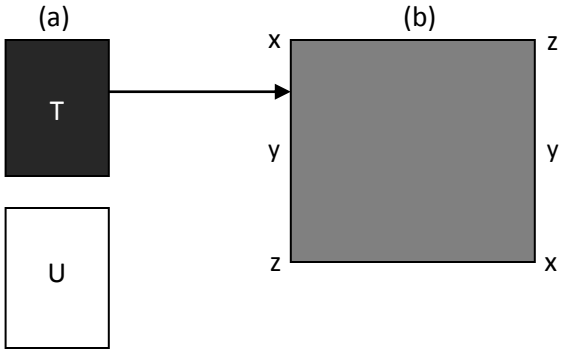

Figure 2. (a) Pair of 2x2-m plots. At random, one plot in each pair was treated with herbicide (T) while the other was left as an untreated control (U). (b) Plot Detail showing soil sampling locations: X (2006), Y (2007), Z (2008).